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# A Study of the Effectiveness of Sulfonamide Preparations in the Elimination of Bovine Mastitis[1]

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## INTRODUCTION

Bovine mastitis is one of the most serious and costly animal diseases with which dairy farmers have to contend. The total monetary losses attributable to this disease through reduced milk production, sale of affected dairy cows for beef, and other factors are not easily measured but they run into millions of dollars annually. The monetary losses are serious at any time, but in wartime the reduction in milk production is especially deplorable.

During the early months of the present war Dr. Adolph Eichhorn, at that time Director of the Animal Disease Station, Bureau of Animal Industry, was invited to England to confer with British livestock authorities with regard to possibilities for increasing their inadequate supply of dairy and other animal food products. After he had reviewed the situation, one of his primary recommendations was that measures be instituted for controlling bovine mastitis in the British herds.

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In the United States also the wartime demand for dairy products has exceeded the available supply. The milk-production goal for 1942 called for an increase of 7¾ percent over the total production for 1941. The increase realized amounted to only 3.2 percent. There were a number of reasons why the goal was not reached but the loss in milk production resulting from mastitis undoubtedly was an important factor.

In a survey (*5*)[3] of more than 300 herds of a typical dairy area in Michigan, it was found that 26.2 percent of the udders of all lactating cows were infected with streptococci—the most common cause of bovine mastitis. Shaw and Beam (*26*) concluded that mastitis infection reduced milk production in affected quarters of the udder by approximately 22 percent. On the basis of these figures the Nation-wide loss in milk production caused by mastitis would amount to nearly 1½ percent (1.44 percent) of the total, if only one quarter of each infected udder were affected. Since cows having mastitis infection are sometimes affected in all four quarters of the udder, it is probable that the average number of affected quarters per infected cow would be at least two. This would increase the percentage reduction or loss in milk production to approximately 3 percent of the total. If the effects of bovine mastitis could have been eliminated in 1942, the prevention of the 3 percent loss in milk production, combined with the 3.2 percent increase actually achieved, would have made it possible to approach the production goal more closely.

But irrespective of goals, 3 percent of the total amount of milk produced in 1942[4] is 3,577,000,000 pounds, or nearly 2 million (1,788,500) tons. This is enough to furnish approximately a pint of milk each day to more than 9 million persons through the entire year. At the conservative estimate of $2 per 100 pounds as the average monetary value of milk at the farm, this would amount to the staggering sum of more than 71 million dollars. This is just one item of the price the dairy farmers of the United States are now paying for having mastitis infection in their herds, as it takes into account only the reduction in milk secretion.

In addition there is the loss of the milk produced by mastitic cows which is unfit for human food. There is the extra labor and expense involved in caring for diseased animals and there is the loss involved in the sale of dairy cows on account of infected udders. In this connection Baltzer (*1*) showed that 11 percent of the cows removed from dairy-herd-improvement association herds in Michigan over a 6-year period were removed because of udder trouble. Another survey (*15*) made by the Bureau of Animal Industry, in which farmers throughout New Jersey gave their reasons for sending their cows to auction markets during a given week in 1943, showed that 49.9 percent of the total were sold on account of mastitis. Estimates of total annual monetary losses in the United States attributable to mastitis run to at least $200,000,000 (*18*).

In October 1942, the Bureau of Dairy Industry began a survey of the extent of mastitis infection in its dairy herd at the Beltsville Research Center, Beltsville, Md., and at the same time began a study to deter-

[3] Italic numbers in parentheses refer to Literature Cited, p. —.

[4] Statistical Report, Milk Cows and Milk Production on Farms, by States, 1942, Bureau of Agricultural Economics, U. S. D. A., Feb. 20, 1943. [Processed.]

mine the effectiveness of sulfonamide preparations in the eradication and control of the disease. The work was undertaken as a wartime project, the object of which was to study methods of eliminating the inhibiting effects of udder infections on milk production and to determine if it is possible or feasible to handle a herd in such a manner as to keep it free of mastitis after the existing infections are eliminated. This bulletin presents the results of the first 10 months' work, and includes all results obtained from the intramammary injections of sulfanilamide-in-oil, and sulfadiazine-in-oil, used singly or in combination, through August 18, 1943, the last date on which these preparations were used.

## REVIEW OF LITERATURE

### Incidence of Mastitis and Nature of Infections

The number of mastitis-infected cows in the United States can be estimated only from the recorded data obtained by surveys in representative areas. Various estimates indicate that more than 85 percent of the herds and from 25 to 75 percent of the cows are infected with organisms capable of causing the disease. In a Michigan survey, Bryan (*5*) found that 86 percent of the herds and 26.2 percent of all lactating cows were infected with streptococci. In a survey in California, Schalm (*24*) found that few herds were free of infection and that from 25 to 75 percent of the cows were infected. Miller and Johnson (*14*) found streptococci in the milk from 47.4 percent of 629 cows in 9 dairy herds. According to Palmer (*18*) surveys in the United States and Europe show that approximately 25 percent of the dairy cattle are infected with mastitis.

Nearly all authorities agree that streptococci are the organisms most commonly associated with mastitis. Palmer (*17*) stated that this group of organisms is considered by some to account for 90 percent of all mastitis. Bryan (*5*) concluded that streptococci were responsible for about 98 percent of all infectious mastitis and that *Streptococcus agalactiae* alone accounted for 95 percent. The percentage of streptococcal mastitis varies greatly in different herds, however. For example, Palmer (*18*) found that staphylococcal mastitis is more important than streptococcal mastitis in some herds. Organisms less frequently associated with mastitis are *Pseudomonas aeruginosa*, coliform bacteria, and *Corynebacterium bovis*. In some herds, serious outbreaks of mastitis result from these less common types of infection. According to Palmer (*18*) cases resulting from these infections usually are acute and treatment is not very satisfactory.

### Prevention, Eradication, and Control

Sanitation, suitable housing, good feeding and management, and proper milking methods have been advocated as preventive measures. Sanitation, segregation, and milking infected cows last in the string, have been recommended as means of preventing the spread of the disease from one cow to another.

Eradication of the disease has been attempted in a variety of ways. Many attempts have been made to eliminate and control the disease by treating only the cows that show clinical symptoms, practicing good

management, and disposing of cows that do not readily respond. Obviously such a procedure does not remove infection from udders that do not show clinical symptoms, and as a result an outbreak is likely whenever predisposing conditions occur.

Inunctions (salves, ointments, etc.) have been used for many years to relieve udder swelling and inflammation. Hot fomentations, applied in various ways, have seemed to be beneficial; in fact, the application of hot water (in a bag in which the udder can be suspended) was considered for years to be one of the most effective means of relieving acute cases of mastitis in the Bureau's herds. Bags for the purpose are on sale by veterinary supply houses. Frequent milking, with massaging of affected quarters, has long been practiced as a means of relieving acute mastitis. Pounden (*20*), on the contrary, reported only temporary relief from frequent milking, but entirely satisfactory results by discontinuing the milking of the affected quarters until the acute inflammatory reaction had subsided. Guard (*9*) reported satisfactory results from the more drastic practice of completely drying up the affected quarters, thus giving them a rest and preventing spread of the infection.

Vaccines have been used with varying degrees of success. Bryan and his associates (*3*, *4*, *6*, *7*) reported certain beneficial results from the use of various vaccines. Studies by Seddon and Rose (*25*) also indicated that vaccines were beneficial. On the other hand Plastridge and his associates (*19*) reported results indicating that vaccination was of doubtful value. Hucker and Hansen (*10*) reached similar conclusions.

In recent months numerous advocates of rapid, short-time milking—particularly when milking machines are used—have stressed the importance of this procedure as a means of preventing mastitis. One of these has pointed out that fewer cases of mastitis occurred in a herd after the rapid milking technique was established than before. However, experimental data on this point do not appear sufficient to justify definite conclusions.

Short-wave diathermy has been used in treating mastitis but results reported do not appear to be conclusive.

One of the most recent recommendations for relieving mastitis is the 5-day fast of affected cows. According to Washburn (*31*) this procedure relieves the symptoms without producing any detrimental effects.

Chemotherapy is playing a prominent role in studies of mastitis control. Various degrees of success have been claimed for udder infusions of acriflavin, entozon, rivanol, trypaflavin, and uberasan. Colloidal silver oxide (Novoxil) has been found to be effective in eliminating udder infections, but unfavorable udder reactions have been reported in some cases. Good results have also been reported from udder infusions of tyrothricin and its derivatives—gramicidin and tyrocidin. Sulfanilamide, given orally, has been used by a number of workers but apparently with varying success.

Reports from various sources have appeared since about 1941 stressing the advantages of mineral oil as a carrier for different preparations used for udder infusion, because of its nonirritating effect on mammary-gland tissues. Little et al. (*12*, *13*), Tripp and Lawrence (*28*), and Edwards (*8*) showed the advantages of oil as a carrier for

gramicidin. Kakavas et al. (*11*) and Palmer (*18*) apparently were the first to report on the use of homogenized sulfanilamide-in-oil and of homogenized sulfathiazole-in-oil for udder infusion. Sanders (*21*, *22*, *23*) reported beneficial results from the use of iodized mineral oil and of sulfanilamide in iodized mineral oil. Some of the manufacturers of biological products are offering preparations of iodized hydrocarbon oil, gramicidin-in-oil, and sulfanilamide-in-oil.

The spectacular results obtained from the use of penicillin and penatin in treating various infections have led to considerable speculation as to its effectiveness in treating mastitis. To date the supply of the purified materials has been so limited that their use in treating animal diseases has been almost prohibitive. It is reported that studies are under way to determine the effectiveness of penicillin in treating mastitis.

Many preparations have proved to be effective in treating streptococcal mastitis, but most of them are reported to be relatively ineffective against staphylococcal infections or other infections associated with mastitis.

## EXPERIMENTAL METHODS AND MATERIALS

### Plan of Study

The size of the Bureau's herd at Beltsville, and the limited facilities of the bacteriological laboratory for handling large numbers of milk samples, made it impracticable to attempt to determine the extent of the udder infection in the entire herd before beginning to treat the udders that were found to be infected. Only 96 milk samples could be conveniently handled in the laboratory at one time; consequently the sampling was begun with a unit of 24 cows. The cows that were found to be shedding organisms associated with mastitis were treated. About 10 days after treatment the cows were again sampled to determine the results of treatments administered, and a sufficient number of unsampled cows was added to the list of treated ones to make up a unit of 24 cows. In this manner animals were added until all cows in the barn had been sampled; after which the sampling proceeded into the next barn, which was handled in a similar manner.

All cows that were moved from other barn units into a sampled area were sampled as soon as possible. Such a procedure was not ideal because of the possibility that cows that were clean at the outset might have become infected before their turn came to be sampled. However, there is nothing in the results to indicate that this was a disturbing factor. All cows were sampled at the beginning of a lactation period, whether previously sampled or not.

Cows not cleared of infection by the first treatment were given additional treatments as soon as practicable. Those found to be free of infection after treatment were not again sampled until the beginning of the next lactation period unless they showed some disturbance such as flakes on strip-cup examination, or acute mastitis. Acute mastitis cases were treated at once without waiting to determine the nature of the infection involved although, when possible, milk samples for bacteriological analysis were obtained before treatment. Cows that were going dry when found to be infected were treated as dry cows. Segregation was not practiced.

## Procedures for Sampling and Laboratory Testing

Milk samples for laboratory examination were taken just prior to the regular afternoon milking. First, the udder and flank of the cow were wiped clean with a cloth wet in a solution containing about 200 p. p. m. of available chlorine. Then, the end of each teat was wiped off with a pledget of cotton wet with alcohol, particular attention being given to cleaning the teat orifice. The first stream of milk was milked into a strip cup and observations were made for flakes. Approximately 4 ounces of the foremilk was then drawn into a sterile 8-ounce jar. Care was taken to prevent any foreign particles from falling into the jar. A separate sample was taken from each quarter of the udder and each sample was marked with the number of the cow and the quarter from which it was taken. The samples were iced and sent to the laboratory the following day for examination.

The laboratory examination consisted of (1) the Hotis test, (2) a microscopic examination of a smear prepared from the completed 24-hour Hotis test, (3) culture plate examination on standard tryptone—glucose—extract—milk agar, and (4) counting the leucocytes by the direct microscopic method.

The standard tryptone—glucose—extract—milk agar was used instead of blood agar in order to save time in the plating procedure. Although the ability of the bacteria to hemolyze blood could not be determined with this medium, it was not difficult, after a little experience, to differentiate fairly accurately between the colony types of the different groups of organisms, such as streptococci, staphylococci, coliforms, and *Pseudomonas aeruginosa*. Representative colonies were transferred into beef infusion broth for identification as to groups but because of lack of time no species identification was attempted.

In the case of infection with *Pseudomonas aeruginosa*, the organisms may be present in any single sample in such small numbers that they do not appear on the plates, yet, with subsequent sampling, they may appear in fairly large numbers. In order to avoid such "spotty" results, an enrichment method was employed when *Ps. aeruginosa* infections were suspected. From the completed 24-hour Hotis test a loopful of the milk was transferred to a tube of beef infusion broth and the tube incubated at 37° C. With this procedure, if *Ps. aeruginosa* is present it will tend to overgrow other organisms that may be present and develop the characteristic fluorescent green color in the broth, from which the organisms may then easily be isolated. Only rarely were false positive results obtained with this enrichment method.

At the initial testing of a cow, the results from the first set of samples were not considered conclusive unless the evidence was so clear-cut as to eliminate all doubt. Such clear-cut results were occasionally found in cases of streptococcal and *Pseudomonas aeruginosa* infections. Whenever any doubt existed a second, rarely a third, set of samples was examined before a final interpretation was made. After a quarter had been found infected and was treated, the presence of the infecting organisms in the first follow-up sample was considered evidence of failure of that treatment. If, however, no evidence of the infecting organisms was found in the first post-treatment sample, a second sample, rarely a third one, was examined. Treatment was

adjudged successful only after at least two consecutive post-treatment samples were found to be free of the infecting organisms.

### Preparations Used in Treating Infected Udders

The studies of Kakavas, Palmer, Hay, and Biddle (*11*) indicated that homogenized sulfanilamide in mineral oil was a highly effective chemotherapeutic agent in the treatment of streptococcal infections in cow udders. The low incidence of physiological reactions resulting from the infusion of this material and the absence of any depressing effect on level of milk production reported by these workers seemed to be decidedly in its favor. The descriptions given in their article for the preparation and administration of this material were used as a general guide and a small supply of a similar preparation was made up.

Meanwhile reports had come to light of extensive studies in the medical field showing that sulfadiazine, a product synthesized by American workers in 1941, was one of the most effective of the large group of available sulfa drugs. According to Palma and Smallwood (*16*) sulfadiazine was the drug of choice when a sulfa drug was indicated, except in gonorrheal infections. Although more slowly absorbed it was more slowly excreted than sulfanilamide, a higher blood concentration could be obtained, and it was by far the least toxic of the sulfa drugs. The evidence shown by Valk (*30*) that sulfadiazine was highly effective in staphylococcal infections and as effective as sulfanilamide in streptococcal infections made it appear particularly attractive for use in treating mastitis—particularly in udders infected with staphylococci. More recently Bryan (*2*), reporting for veterinary medicine, voiced similar conclusions to the effect that sulfadiazine is less toxic and more easily tolerated than the other sulfa drugs in veterinary practice and as effective when used according to recommendations.

Some difficulty was experienced in obtaining a supply of sulfadiazine for experimental use because of the requirements of the armed forces. The cost was nearly 10 times as great as that of sulfanilamide. It was homogenized in mineral oil in essentially the same way as the sulfanilamide, except that a larger proportion of oil was required to provide a material having a workable consistency. In view of the scarcity and the cost the sulfadiazine was used sparingly.

Except for a very few quarters treated at the beginning of the work, when sulfadiazine-in-oil was used alone in small doses (20 cc.), the sulfadiazine was used in a mixture prepared by adding a stock preparation of sulfadiazine-in-oil to preparations of sulfanilamide-in-oil in such a proportion that the mixture contained sulfanilamide and sulfadiazine in the ratio of approximately 19 to 1 (95 and 5 percent, respectively). For purposes of brevity in subsequent discussions of techniques and results, the sulfanilamide-in-oil will be referred to as S, the sulfadiazine-in-oil as SD, and the preparation containing both drugs as S+SD.

As the work progressed various methods of mechanical mixing and homogenizing were used. Oils of different viscosities and different proportions of oil and drug were used in an effort to increase the

stability and facilitate the administration of the product. The products were sterilized in glass containers suspended in a hot water bath, at a temperature of approximately 65° to 70° C. for 2 hours. Some degree of separation occurred when the preparations were allowed to stand for several hours, but a homogeneous material was readily obtained by shaking before using.

## Administration of Treatment

### TECHNIQUE

Except in unusual cases only the quarters known to be infected were treated. Ordinarily treatment was given once a day on 4 successive days, although in a few cases treatment was given twice daily for 3 successive days. In lactating cows injections were made as soon as possible after milking, and the material was left in the udder until the next regular milking. The milk containing the residue of the injected preparation was discarded. In dry cows the injections usually were made on 4 successive days and all of the injected material was left in the udder.

The stock material was thoroughly agitated before commencing treatment in order to insure uniform distribution of the sulfa drugs throughout the mixture. Usually a small quantity was transferred to a sterilized can with a lid, to permit easier handling and more complete dispersion of the suspended ingredients by agitation. If a number of cows were being injected, the can was set in a bucket of hot water in order to maintain a temperature that would facilitate easy handling.

A 50-cc. glass and metal serum syringe with Luer type nozzle fitting was used. The injection cannulas should have as large a bore as possible, and still allow easy access into the teat canal. A sterilized cannula was used for each quarter treated. Before use the cannulas were kept immersed in a 50-percent alcohol solution or wrapped in cotton moistened with alcohol solution. Following the injection the quarter was massaged upward for the purpose of distributing the injected material through the cistern and into the larger milk ducts. It was found desirable to disassemble the syringe after each day's use, to wash the oil residues from all parts—especially the rubber plungers—and to keep the syringe and plungers in an alcohol solution when not in use.

### DOSAGE

At the outset of the study, 40 cc. of the sulfanilamide-in-oil (S) was given as a daily dose for each infected quarter. The few quarters treated with sulfadiazine-in-oil (SD) received 20 cc. as a daily dose. Some difficulty was experienced in administering the sulfanilamide preparation because of its heavy consistency so the proportion of mineral oil to sulfanilamide was increased slightly and the dosage was raised to 50 cc. On the basis of the quantity of material obtained from a given quantity of ingredients used in preparation, it was calculated that a 50-cc. dose of the more fluid material contained 13.5 gm. of sulfanilamide. This was slightly less than the quantity of sulfanilamide per dose recommended by Kakavas et al. (*11*).

Later, the dosage was increased. Some of the infected quarters that failed to respond to repeated treatments of 50-cc. injections, were given substantially heavier doses, some up to 150 cc. per day for 4 successive days. Probably more infections would have been eliminated with one treatment, had the initial dosage been not less than 75 cc. per day.

## DISCUSSION OF RESULTS

### Extent and Nature of Mastitis Infection in the Beltsville Herd

A total of 185 cows in the Bureau's breeding herd at Beltsville were examined for udder infection, but the data obtained for 9 of them were incomplete and are omitted from this discussion.

Of the 176 cows for which satisfactory data were obtained, 115 were found to be free of udder infection. The other 61 were infected in one or more quarters. Four types of organisms were found to be present: Streptococci (mostly *Streptococcus agalactiae*), staphylococci, *Pseudomonas aeruginosa*, and coliform bacteria.

The 61 infected udders contained 134 infected quarters—an average of 2.2 quarters to the udder. Of the 134 infected quarters 97 quarters (72.4 percent) contained streptococci, 12 quarters (9.0 percent) contained staphylococci, 19 quarters (14.2 percent) contained *Pseudomonas aeruginosa*, and 6 quarters (4.5 percent) contained coliform bacteria.

Some of the cases of infection were discovered only a short time before it was decided to change to the use of another sulfonamide preparation and were not treated with any of the preparations described (S, SD, or S+SD). Nine such cases are included in the 134 infected quarters. Six of these contained streptococci, two contained staphylococci, and one was infected with *Pseudomonas aeruginosa.*

This summary, therefore, is based on the results of treating 125 quarters, 91 of which were infected with streptococci, 10 with staphylococci, 18 with *Pseudomonas aeruginosa*, and 6 with coliform bacteria.

### Effectiveness of Sulfonamide Therapy

Table 1 shows the effectiveness of sulfonamide therapy in treating 125 udder quarters that were infected with various types of mastitis organisms.

Table 1.—*Effectiveness of sulfonamide therapy (sulfanilamide and sulfadiazine separately or in combination) in treating 125 udder quarters infected with various types of mastitis organisms*

| Infecting organism | Quarters treated | Treated quarters cleared | |
|---|---|---|---|
| | *Number* | *Number* | *Percent* |
| Streptococci | 91 | 73 | 80.22 |
| Staphylococci | 10 | 9 | 90.00 |
| *Pseudomonas aeruginosa* | 18 | 10 | 55.55 |
| Coliform bacteria | 6 | 5 | 83.33 |
| All types | 125 | 97 | 77.60 |

In view of the lower percentage of favorable results obtained from treating quarters infected with *Pseudomonas aeruginosa* as compared

with those obtained from treating quarters infected with the other organisms, it is noteworthy that four of the eight failures were associated with acute mastitis. Furthermore four of those that did not respond were listed as failures although they had received only one treatment. In two of these four cases the cows went dry and were slaughtered, and in the other two the sulfonamide preparation [5] was changed after the first treatment.

In referring to the number of treatments required to eliminate an infection, or the number of treatments preceding a failure, a series of daily injections—usually four on consecutive days—is considered as one treatment.

By way of contrast only 4 of the 18 failures to eliminate streptococcal infections were classed as failures after 1 treatment. In 2 of these the cows affected were killed, and in the other 2 a change in the sulfonamide preparation was made after 1 treatment had been given. Eleven of the 18 quarters received 5 or more treatments. Only 1 of the 18 was associated with acute mastitis.

Only 1 of the 10 quarters infected with staphylococci failed to respond to treatment. It was treated 3 times. It was a chronic case. Likewise, only 1 of the 6 coliform-infected quarters failed to respond. This case was not acute, and the failure was based on a single treatment administered before changing to another sulfonamide preparation.

There is little likelihood that the percentage of quarters cleared would have been substantially raised by continuing treatment in the quarters infected with streptococci or staphylococci. On the other hand it appears that the results might have been more favorable in the quarters infected with coliform bacteria and with *Pseudomonas aeruginosa* if the same treatment had been continued for a longer time.

### Number of Treatments Required to Eliminate Udder Infections

The number and percentage of the 97 cleared quarters that responded (became cleared of infection) as a result of the first, second, third treatment, and so on, are shown in table 2.

None of the 24 quarters that were cleared of staphylococci, *Pseudomonas aeruginosa*, or coliform bacteria required more than 3 treatments to eliminate the infection. Of the 73 quarters that were cleared of streptococci, only 6 required more than 3 treatments. Despite the persistency of some of the streptococcal infections, approximately 92 percent of those that finally responded had been cleared by the first, second, or third treatment.

With the exception of the *Pseudomonas aeruginosa* infections, from 60 to 66.67 percent of the quarters that finally responded were cleared as a result of the first treatment. For all types of infection combined, 67.01 percent of those that responded were cleared by the first treatment, 86.60 percent by the first or second treatment, and 93.81 percent by one of the first three treatments. It appears from these results that, for those quarters that have not cleared as a result of three treatments, the prognosis is not favorable although a few may respond to subsequent treatments.

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[5] Results obtained from the use of the new preparation are not included in this tabulation. This report is based on the results obtained with sulfanilamide and sulfadiazine, used singly or in combination.

TABLE 2.—*Number of treatments by sulfonamide therapy required to eliminate organisms of infection from the 97 quarters that were cleared*

| Infecting organism, number of quarters treated, and sequence of treating | Quarters cleared by each treatment | | Cumulative proportion of quarters cleared |
|---|---|---|---|
| | *Number* | *Percent* | *Percent* |
| Streptococci (73 quarters): | | | |
| First treatment | 47 | 64.38 | 64.38 |
| Second treatment | 17 | 23.29 | 87.67 |
| Third treatment | 3 | 4.11 | 91.78 |
| Fourth treatment | 3 | 4.11 | 95.89 |
| Fifth treatment | 1 | 1.37 | 97.26 |
| Sixth treatment | 1 | 1.37 | 98.63 |
| Seventh treatment | 0 | 0 | 98.63 |
| Eighth treatment | 1 | 1.37 | 100.00 |
| Staphylococci (9 quarters): | | | |
| First treatment | 6 | 66.67 | 66.67 |
| Second treatment | 2 | 22.22 | 88.89 |
| Third treatment | 1 | 11.11 | 100.00 |
| *Pseudomonas aeruginosa* (10 quarters): | | | |
| First treatment | 9 | 90.00 | 90.00 |
| Second treatment | 0 | 0 | 90.00 |
| Third treatment | 1 | 10.00 | 100.00 |
| Coliform bacteria (5 quarters): | | | |
| First treatment | 3 | 60.00 | 60.00 |
| Second treatment | 0 | 0 | 60.00 |
| Third treatment | 2 | 40.00 | 100.00 |
| All types (97 quarters): | | | |
| First treatment | 65 | 67.01 | 67.01 |
| Second treatment | 19 | 19.59 | 86.60 |
| Third treatment | 7 | 7.22 | 93.81 |
| Fourth treatment | 3 | 3.09 | 96.91 |
| Fifth treatment | 1 | 1.03 | 97.94 |
| Sixth treatment | 1 | 1.03 | 98.97 |
| Seventh treatment | 0 | 0 | 98.97 |
| Eighth treatment | 1 | 1.03 | 100.00 |

Although only 55.55 percent of the quarters infected with *Pseudomonas aeruginosa* were cleared of the infection, it is noteworthy that 90 percent of the quarters that were cleared responded to the first treatment. It appears that if this organism is not eliminated by the first treatment, it is eliminated only with great difficulty. Some organisms build up a resistance to sulfa drugs. Possibly *Ps. aeruginosa* reacts in this manner to moderate doses of sulfonamides. It may be that heavier initial doses would have cleared a higher percentage of infected quarters.

As stated, some bacteria possess or acquire strong resistance to sulfonamides. Probably, too, as Sanders (*21*) has pointed out, the results may depend on the location and extent of the infection—the foci in or near the cistern being more accessible and offering more favorable opportunity for successful treatment than foci situated in the upper glandular tissues. It is not unlikely that the location of the infection, as well as changes in the tissues, such as the formation of abscesses or other lesions, may account to a large extent for the few "incurable" cases that are reported in connection with most forms of treatment. These factors also may account for the wide variation in number of treatments required to eliminate the infection in udders that finally respond.

## HOT WATER TREATMENT (FOMENTATION) IN CONJUNCTION WITH SULFONAMIDE THERAPY

The application of heat to the udders of cows suffering from acute mastitis has been practiced for a number of years and has proved

effective in relieving the acute symptoms of the disease. Special bags are manufactured and sold which can be fastened to the cow in such a manner that the udder can be suspended in a hot water bath for as long a time as may be desired.

In a few cases of persistent infections, and in one case of acute mastitis, a combination of the hot water bath and the sulfonamide injections was tried. Water at temperatures of 108° to 110° F. was maintained in the water bag in direct contact with the udder for periods of 40 to 60 minutes each day, commencing immediately after each of the daily injections of sulfa drugs.

This treatment was given to 16 quarters of 8 cows. One of the cows received the hot water treatment in conjunction with two treatments. Twelve of the quarters were infected with streptococci, 7 of which had failed to respond to from 4 to 6 sulfonamide treatments. Only 2 of the 12 streptococcus-infected quarters were found to be free of infection after the combined treatment was given. Two staphylococcus-infected quarters were given the combined treatment. One of these responded and 1 failed to respond. The combined treatment was given also to 2 quarters infected with *Pseudomonas aeruginosa*. Both cases failed to respond. One had previously been treated 5 times without success. The other was an acute case with no previous treatment. It occurred in a cow that was in her third lactation period and that had no previous history of mastitis. The cow became steadily worse and milk secretion stopped entirely. She was slaughtered 73 days after the onset of the acute symptoms. Post-mortem study showed an enormous abscess and extreme tissue destruction in the affected quarter.

One of the 16 quarters that was given hot water fomentations in conjunction with 2 separate series of sulfonamide injections received 150 cc. at each daily injection during the second series. It failed to respond. The cow with acute mastitis (*Pseudomonas aeruginosa*) received 50 cc. at each daily injection. All of the others received 100 cc. at each daily injection.

Although many of the infected quarters that were given this treatment had failed to respond to prior treatments, the percentage of infected quarters (19 percent) responding to the combined sulfonamide and hot water treatment is not impressive.

### Effect of Injecting Sulfa Drugs More Frequently and for a Shorter Period of Time

On the basis of reports that experience had led some practitioners to believe that injecting sulfonamides more frequently than once daily and shortening the duration of treatment was beneficial, 6 cows were treated twice daily with 100 cc. of sulfa drugs, for 3 successive days. Of the 11 quarters treated in this manner, 10 were infected with streptococci and 1 with *Pseudomonas aeruginosa*. The dosage at each injection was 100 cc.; thus each quarter received 200 cc. daily and 600 cc. for the series of injections. All but 2 of the 11 quarters had been treated previously. Eight had received from 2 to 7 previous treatments. In the case of 6 quarters, the previous treatment consisted of hot water fomentations in conjunction with sulfonamide injections, 1 having had 2 such treatments. This information is given to show that a high percentage of these quarters had persistent

infections. The only quarter that responded to the intensified treatment had previously received 7 series of injections of sulfa drugs, 2 of which series had been accompanied by hot water fomentations. The data are insufficient to provide proof that the more intensified form of treatment was advantageous.

## Relative Effectiveness of Sulfanilamide and of Sulfanilamide and Sulfadiazine

A tabulation was made in an attempt to determine whether or not the sulfanilamide with sulfadiazine added (S+SD) was more effective than the sulfanilamide alone (S). Such a tabulation would lend itself to a more nearly accurate evaluation of the relative merits of the two preparations if it had been feasible at the outset to set up the project on a strictly experimental basis with separate groups of animals having infections of like nature and equal severity and resistance, and with each group receiving one sulfonamide preparation exclusively. Unfortunately it was not feasible to conduct such an experiment.

A summary of the results obtained from the use of S alone and from S+SD is given in table 3. In order to simplify the comparisons the one quarter that received SD alone for 4 days and the three quarters that received SD for 1 day and S for 3 days were included in the S+SD group.

Table 3.—*Comparison of results obtained with sulfanilamide-in-oil (S) and with sulfanilamide-in-oil containing sulfadiazine (S+SD) in treating infected quarters*

| Treatment and infecting organism | Quarters treated | Treated quarters cleared | |
|---|---|---|---|
| Sulfanilamide (S): | *Number* | *Number* | *Percent* |
| Streptococci | 49 | 36 | 73.47 |
| Staphylococci | 2 | 1 | 50.00 |
| *Pseudomonas aeruginosa* | 8 | 4 | 50.00 |
| Coliform bacteria | | | |
| All types | 59 | 41 | 69.49 |
| Sulfanilamide and sulfadiazine (S+SD): | | | |
| Streptococci | 55 | 37 | 67.27 |
| Staphylococci | 9 | 8 | 88.89 |
| *Pseudomonas aeruginosa* | 13 | 6 | 46.15 |
| Coliform bacteria | 6 | 5 | 83.33 |
| All types | 83 | 56 | 67.47 |

The high effectiveness of S+SD in the treatment of staphylococcal and coliform infections is shown by table 3. It appears that preparations containing sulfadiazine are highly effective, especially against staphylococcal and coliform infections, although the number of quarters treated with sulfanilamide alone is too small to provide a satisfactory comparison of results.

For streptococcal and pseudomonas infections and for all types of infections combined, the comparisons shown in table 3 might be interpreted as indicating that the S+SD preparation was slightly less effective than S alone. However, a number of factors must be taken into account in interpreting these results:

1. In many cases treatment with S alone was followed by treatment with S+SD. In some of these cases treatment with S alone was less persistently followed than was the case with S+SD. That is, the change from S to S+SD may have been made after a single treatment with S, in which case a failure would be charged against S.
2. Treatments with S+SD, on the other hand, were begun after many quarters had been cleared with S alone, thus the percentages based on S+SD include the more persistent cases.
3. All cases that had not responded to treatment with S+SD when the use of still another preparation was begun, are counted as failures for S+SD, even though they may have responded to the new treatment subsequently.

Eighteen quarters of 11 cows were treated with S+SD after failure to respond to S alone. Thirteen were infected with streptococci, 1 with staphylococci, and 4 with pseudomonas organisms. Five of the quarters infected with streptococci, 1 infected with staphylococci, and 1 with a pseudomonas infection, responded to the S+SD injections. Thus 7 of the 18 infected quarters that had failed previously when treated with S alone were cleared with S+SD.

Since treatment with S+SD included a considerable number of quarters with persistent infections that previously had failed to respond to treatment with S alone it would seem that S+SD probably is equally as effective as S against streptococcal and pseudomonas infections, and definitely superior in the case of staphylococcal infections. A high degree of effectiveness is shown for S+SD against coliform infections although comparative data cannot be given.

In view of the fact that Kakavas et al. (*11*) had reported that staphylococcal infections were not readily eliminated by sulfanilamide-in-oil except with the use of large doses, the high effectiveness of the preparations containing sulfadiazine is especially significant. Kakavas et al. (*11*) also reported that sulfathiazole was objectionable because it caused the formation of hard curds of casein, difficult to remove, which temporarily plugged the teat duct. No difficulties of this nature were noted following the use of preparations containing sulfadiazine. In fact no objectionable reactions were found to occur.

### Results Obtained in Treating Acute Mastitis With Sulfa Drugs

During the period of 10 months covered by this report acute mastitis occurred in a number of cows. Results of treatment are available for acute mastitis in 14 quarters involving 9 cows. Five quarters were infected with streptococci, 1 with staphylococci, 6 with pseudomonas organisms, and 2 with coliform bacteria. Four of the 5 streptococcus-infected quarters (80.0 percent), the 1 staphylococcus quarter (100.0 percent), 2 of the 6 pseudomonas quarters (33.3 percent), and both of the coliform infected quarters (100.0 percent) were cleared of the infecting organisms. Thus 9 of the total 14 quarters (64.3 percent) were cleared of infection by sulfonamide injections. The results for the various types of infections resulting in acute mastitis do not differ greatly from the combined results based on treatments for all cases (table 1).

During the same period 10 quarters of 5 other cows were affected with acute mastitis and received treatment but either the nature of the

infection was not determined or the results were inconclusive; hence the data are not included.

### Effectiveness of Treatment in Dry Cows

Most investigators have reported better results from treating cows during the dry period than from treating during lactation. The cows in this study were treated as soon as possible after the presence of the infection was known; consequently most of them received treatment during lactation. Eight quarters of five cows were treated during the dry period. All of the eight were infected with streptococci. Three of the quarters cleared and five failed. However, the low percentage of quarters that cleared should not be considered as an indictment against the treating of dry cows because of the persistent nature of the infection in the five quarters that failed. For example, four of the five quarters that failed had received four previous treatments in which S, S+SD, and S+SD in conjunction with hot water fomentations were used. The other quarter had been treated six times while the animal was lactating—three times with S alone and three times with S+SD.

### Effect of Sulfonamide Treatments on Milk Production

An important question in connection with treatments for mastitis in lactating cows is the effect on milk production. Records are available for 50 cows in 53 lactation periods. Sulfa-drug treatments included both S alone and S+SD. They were given at all stages of lactation and at greatly varying lactation levels, and they involved from 1 to 4 quarters of the udder. Some of the cows were in early lactation when a rise in milk production might have been expected; others were in very late lactation and drying off rapidly. The 10-day pretreatment production averages for individual cows ranged from 15.37 pounds to 83.95 pounds of milk daily. Because an upset physical condition and a depressed milk production are likely to have occurred before acute mastitis was detected, none of the cases involving treatment for acute mastitis are included in these comparisons. Details of the changes in production are given in table 4.

Table 4.—*Effect of sulfonamide treatments on milk production*

| Stage of lactation when treatment commenced | Lactation periods [1] | Average daily milk production | | | Increase (+) or decline (−) in milk production by— [2] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cows treated in— | | | | All cows |
| | | 10 days before treatment | 4 days during treatment | 10 days after treatment | 1 quarter | 2 quarters | 3 quarters | 4 quarters | |
| | *Number* | *Pounds* | *Pounds* | *Pounds* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Under 3 months | 27 | 44.58 | 42.81 | 42.48 | −1.85 | −7.37 | −12.41 | −8.25 | −4.71 |
| 3 to 6 months | 9 | 23.99 | 23.99 | 24.41 | +.61 | −.02 | +6.05 | ------- | +1.74 |
| 6 to 9 months | 10 | 26.62 | 24.86 | 24.81 | −2.78 | −13.69 | −5.37 | ------- | −6.80 |
| Over 9 months | 7 | 25.00 | 22.90 | 23.95 | +.27 | −1.13 | −7.09 | −10.40 | −4.20 |
| Average or total for all lactations | 53 | 35.11 | 33.60 | 33.63 | −1.51 | −7.13 | −6.25 | −9.27 | −4.21 |

[1] Representing 50 cows, 3 of which were treated in 2 different lactation periods.
[2] Based on a comparison of the average production after treatment with the average before treatment.

The average decline in milk production for the 53 lactation periods—from 35.11 pounds for the 10-day period prior to treatment to 33.63 pounds for the 10-day period following treatment—was 4.21 percent. The time from the midpoint of the pretreatment period to the midpoint of the post-treatment period was 14 days. Except for cows in the early stages of lactation the expected decline in milk production averages about 8 percent per month. The reduction of 4.21 percent in these treated cows during the approximately half-month period does not, therefore, appear to be significantly greater than normal.. However, there was a tendency for the decline in production to be accelerated as the number of treated quarters of the udder increased.

For the entire group of 53 lactation period comparisons involving 50 different cows, the production was lowered in 32 cases, raised in 20 cases, and unchanged in 1 case. Some of the increases and decreases were too small to have any significance.

### Experiments With Other Sulfonamide Preparations and Present Status of Mastitis in the Herd

Attention has been called to the fact that certain strains of bacteria possess, or acquire through contact, a high degree of resistance to sulfonamide preparations. Recent reports (*27*, *29*) brought out the fact that urea increases the solubility of sulfonamides, enhances their bacteriostatic action, and removes fastness of some sulfonamide-resistant organisms.

After considerable experimentation to determine the concentration of urea that could be tolerated by normal mammary tissues, a preparation containing sulfanilamide and urea was made up for use in treating mastitis—especially cases that had failed to respond to sulfanilamide or sulfanilamide and sulfadiazine—to determine the merits of urea in sulfonamide preparations. This has been used in all cases that have required treatment since August 1943. It is the preparation to which reference has been made a number of times in this bulletin as the new sulfonamide which followed the S and S+SD treatments. The results to date appear to be promising. Details will be given in a later report.

In general the situation with respect to the elimination of mastitis from the herd is encouraging. A few cases have failed to respond to any of the sulfonamide preparations used, and these animals have been or will be removed from the herd. A few have cleared only after repeated injections with one or more of the preparations. Nearly all of the cows are coming through their first lactation without mastitis infections, and only a few cows in the herd are known to harbor infections. Flakes on strip-cup examinations are rare.

At present there are four cows in the herd that do not respond to treatment. They will be disposed of. The results of treating seven additional cows have not been determined. Thus it appears that the herd will be free of infection at an early date. The problem then will be to determine whether the herd can be kept free of infectious mastitis by feasible methods of management.

## SUMMARY AND CONCLUSIONS

Because mastitis interferes with milk secretion its control is of great importance, especially in wartime when there is need for increased quantities of dairy products to meet the demand.

During the first 10 months of a study of the eradication and control of mastitis in the herd of the Bureau of Dairy Industry at Beltsville, Md., 61 of 176 cows studied were found by bacteriological tests of milk samples to be infected in one or more quarters of the udder. There were 134 infected quarters—an average of 2.2 quarters to each infected udder. Of the 134 infected quarters 72.4 percent contained streptococci, 9.0 percent staphylococci, 14.2 percent *Pseudomonas aeruginosa*, and 4.5 percent coliform bacteria.

One hundred twenty-five infected quarters received treatment with sulfanilamide-in-oil (S) and sulfadiazine-in-oil (SD), singly and in combination. The organisms were eliminated from 80.22 percent of quarters infected with streptococci, 90.00 percent of those infected with staphylococci, 55.55 percent of those containing *Pseudomonas aeruginosa*, and 83.33 percent of those infected with coliform bacteria. For all types of infections the effectiveness was 77.60 percent.

Of all the quarters that became cleared of infection as a result of treatment, none of those infected with staphylococci, pseudomonas organisms, or coliform bacteria persisted beyond the third treatment, although several of the streptococcal infections responded to later injections. For all types of infections 67.01 percent of all the quarters that became cleared responded to the first treatment, 86.60 percent responded as a result of two treatments, and 93.81 percent as a result of three treatments. Despite the low effectiveness of the sulfonamide preparations used in treating pseudomonas organisms, 90 percent of the quarters that were cleared responded to the first treatment. Apparently this organism is destroyed easily or else with difficulty.

Hot water fomentations were used in conjunction with sulfonamide injections in a number of the more persistent cases. The percentage of cases that responded was not impressive and the added value to be derived from using the two forms of treatment in conjunction is doubtful.

The data are insufficient to provide proof that increasing the frequency and shortening the duration of time covered by the injections was advantageous.

The percentage of total quarters cleared was very slightly lower for S+SD than for S alone, but the S+SD preparation was used in treating many quarters that had failed to respond to injections of S alone. Nearly 40 percent of the quarters that had failed on S alone and that were subsequently treated with S+SD were cleared of infection.

Preparations containing sulfadiazine were very highly effective in treating quarters infected with staphylococci and coliform bacteria.

Unfavorable reactions to sulfonamide injections were extremely rare; in fact almost nonexistent. The preparations containing sulfadiazine did not cause the formation of curds with resulting teat occlusion, a condition reported by others (*11*) in connection with the use of sulfathiazole.

In the treatment of 14 quarters affected with acute mastitis of varying degrees of severity, 9 (64.3 percent) were cleared of infection. All 4 of the infecting organisms on which the results of this study are based were present in one or more of these acute mastitis cases which responded to treatment. Pseudomonas infections were responsible for a high percentage of the acute cases of mastitis in this herd during the period covered by the summary. The effectiveness of sulfonamide therapy was particularly low in treating acute cases involving pseudomonas infections. The results were essentially the same for acute as for chronic mastitis in the case of the other organisms.

Data on the treatment of dry cows are too limited to provide a comparison of the relative effectiveness of treating during the lactating period and during the dry period.

Comparisons in 53 lactation periods of 50 cows that received treatment with sulfonamide preparations show that the average daily milk production for a 10-day period immediately following treatment was only 1.48 pounds (4.21 percent) lower than the average for the 10-day period immediately preceding treatment. These comparisons included cows whose average daily milk production ranged from 15.37 to 83.95 pounds and cows in all stages of lactation. Production was lowered in 32 cases, raised in 20 cases, and unchanged in 1 case. Many of the changes were too small to have any significance. An average decline of 4.21 percent is little if any greater than might be expected in untreated cows during a half-month period. The administration of these sulfonamide preparations apparently did not have a significant depressing effect on milk secretion.

The high degree of effectiveness of the sulfonamide preparations used (especially those containing sulfadiazine) in treating staphylococcal infections, the rarity of unfavorable reactions, and the lack of any significant depressing effect on milk secretion resulting from administration of the treatments indicate the high merits of these preparations for use in eliminating mastitis infections—particularly streptococcal, staphylococcal, and coliform infections.

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